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Procedia Food Science 1 (2011) 1821 – 1827
Procedia Food Science

11th International Congress on Engineering and Food (ICEF11)

Formulation of banana aroma impact ester in water-based microemulsion nano-delivery system for flavoring applications using sucrose laurate surfactant

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Abstract

This investigation aims to formulate a dilutable water-based microemulsion nano-delivery system that can deliver flavor model ester to food or beverage without using organic solvents. Isoamyl acetate (IAA) was used as a simple model of hydrophobic flavoring ester that can impart banana aroma and flavor. Food-grade sucrose laurate was used as non-ionic surfactant at concentration of 5.0% (w/w). Different aqueous phases were used to formulate the microemulsions including pure water and aqueous solutions containing citric acid with sucrose, fructose and glucose, which are frequently used in food and beverage industries. The oil titration method was used to formulate the IAA-in-water microemulsions. Results indicated that sucrose laurate at 5.0% can form micelles having a particle size 1.3nm-2.1nm depending on the sugar composition of the aqueous micellar solutions. Pure water micellar solution was able to solubilize the largest load of IAA (110µl/10g micellar solution ~ 1.434 mmol/g surfactant) in the form of water continuous microemulsion. This value was declined to 80 µl, 70 µl and 60 µl (~1.044, 0.912 and 0.783 mmol/g surfactant) when the aqueous micellar solutions were composed of water/citric/sucrose, water/citric/fructose and water/citric/glucose, respectively. The particle size of all formulated microemulsions at the largest solubilized load of IAA ranged from 4.27nm to 6.24 nm depending on the sugar composition of the aqueous phase. All formulations possessed a potent banana aroma and can be diluted with the corresponding aqueous phase up to 10- folds without losing their microstructure. The formulated microemulsions can be considered as basic nano-delivery models that can potentially be developed and rounded-up by addition of more aroma impact constituents to suite different applications in food and beverage flavoring industry.

Selection and/or peer-review under responsibility of 11th International Congress on Engineering and Food (ICEF 11) Executive Committee.

Keywords: nano-delivery system; microemulsion; flavor; sugars; food applications.

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doi:10.1016/j.profoo.2011.09.267

1. Introduction

Aroma compounds are group of organic volatiles synthesized naturally during fruits ripening via enzymatic reactions. Esters are an important class among these aromatic compounds because they give characteristic aroma impact to many fruits. Food and beverage industries have used combinations of these esters to flavor ice creams, candy, showing gums, artificial juices and nectars and even fruit-flavored soft drinks. Pharmaceutical industry usually use fruit flavors to mask the unpleasant taste of some medications. The hydrophobic nature of most of these esters hampers their delivery to food and beverage in water-based formulas. Thus edible permitted organic solvents, like ethyl alcohol, propylene glycol, triethyl citrate and triacetin are usually used as a vehicle for flavor delivery [1]. Application of these solvents is accompanied by some risks and difficulties in handling, storing and transporting huge amounts of these flammable organic solvents. Health organizations, environmental agencies and some national denominations call for reduction or even elimination of these organic solvents in foods and beverages. Thus developing water-based flavor formulation that satisfy consumers and processors demands becomes indispensable. Microemulsions are promising stable water-based colloidal delivery systems capable of solubilizing poor water-soluble flavors for food and pharmaceutical applications [2]. Sucrose fatty acid esters are among the non-ionic, safe and approved surfactants in many countries to formulate food-related microemulsions [3, 4]. In the present work, the authors aimed to formulate water-based isoamyl acetate (IAA) microemulsion delivery system using sucrose laurate (SL) surfactant. This ester has a potent ripe banana-like aroma that makes it an excellent "simple" model for banana flavor [5]. The effect of added sugars to the aqueous phase on the maximum solubilized load of IAA, particle size and size distribution of microemulsion was studied. The dilutability of the formulated microemulsions was also investigated.

2. Materials & Methods

D-(+)-Glucose (99.5%) and D-(-)-Fructose (99.0%) were supplied from Sigma-Aldrich, Co. St. Louis, US. Sucrose was purchased from the local market. Citric acid and sodium nitrite was supplied from Fisher Scientific UK Limited. Isoamyl acetate (>97.0 %) was supplied from SAFC Supply Solutions (Germany), Sucrose laurate (Ryoto Sugar Ester L-1695, HLB 16) was donated from Mitsubishi –Kagaku Foods Corporation, Japan. It contains more than 90.0% of the ester classified according to the manufacturer as: sucrose monolaurate (78.0-81.0%), sucrose dilaurate (14.0-15.0%) and sucrose tri- and higher laurate (less than 3.0%).

2.1 Preparation and characterization of microemulsions:

The oil titration method was used to prepare the different microemulsions. Five percent (w/w) sucrose laurate (SL) surfactant was dissolved in four different aqueous solutions with or without added sugars as in table (1) to form micellar solutions. The composition of these solutions was based on a model fruit juice (without surfactant) that was previously reported [6] with some modifications. The different transparent micellar solutions of SL were left at room temperature after preparation to equilibrate for one hour. Batches of ten grams of each of the four micellar solutions were placed into a number of glass vials hermetically closed with press caps. Escalating volumes of IAA was titrated into each set of vials starting from 20μl to 150 μl with increment of 10 μl using calibrated automatic micropipette. The same titration procedure was conducted for a parallel groups of the four aqueous solutions formulated without SL surfactant to act as blank controls. The volume of IAA just before the one which caused the micellar solution to become irreversibly turbid after 1 week of equilibration is denoted as the maximum solubilized load to the nearest 10 μl. No attempts to investigate lower volumes than 10 μl because the sensitivity of the micropipette may not be accurate at these low volumes. Microemulsions were characterized by detecting their fluidity and absence of gel phases or birefringence using polarized light microscopy (Leica Model DM 2500M, Leica Microsystems). The nanometer-size characterizing microemulsion was revealed using dynamic light scattering instrument Nano-S (Nano-series, Malvern Instruments, UK). Measurements were done at 25^{o}C with a fixed angle of 90º.

Table 1. Composition of the different aqueous solutions used for formulating IAA microemulsion nano-delivery systems.

Composition of the aqueous phases*	pH	Viscosity (Cp)	Refractive index
Water pure	5.99	7.41	1.338
Water (100g), sucrose (11g), citric acid (0.8g)	2.31	9.045	1.360
Water (100g), glucose (11g), citric acid (0.8g)	2.22	8.110	1.357
Water (100g), fructose (11g), citric acid (0.8g)	2.27	7.82	1.353

*: Containing 5.0g SL surfactant/100g water. All aqueous phases contained 0.2 wt% sodium nitrite as preservative

Sizes quoted are the z-average mean for the microemulsion hydrodynamic diameter (nm). The refractive index of the different microemulsions was measured on Abbe ED/60 precision refractometer (Bellingham and Stanley, Sevenoaks, UK). The viscosity was measured at 25ºC using High Resolution CVOR Bohlin controlled stress rheometer (Malvern instruments limited, Worcestershire, UK). The concentric cylinder measuring system (C25DIN53019) consists of rotating bob (inner cylinder) located in a fixed cup (outer cylinder) with the sample contained in the annular gap between them. The diameters of the cup and bob were 27.5 and 25.0 millimetre (mm) respectively.

3. Results & Discussion

From figure (1) it is evident that SL surfactant can form micelles in the different aqueous solutions at zero load of IAA. The particle size of these empty micelles ranged between 1.3nm-2.1nm depending on sugar type of the aqueous solution. The poly-dispersibility index (PDI) of micelles was mono-modal and equals to 0.1 (data not shown) indicating a uniform micelle size distribution. Gradual titration of IAA into each aqueous micellar solution resulted in spontaneous and fast formation of O/W microemulsion. That was revealed visually by observing the transparency and fluidity of the system during formulation and after equilibration. All microemulsion delivery systems possessed the distinct IAA aroma which is typically reminiscent to banana. Figure (1) showed the gradual increase in micelle size (micelle swallowing) as a function of increasing the solubilized load of IAA. The size increased continuously until reaching the upper limit of solubilization, i.e. the maximum solubilized load, beyond which the microemulsion collapse into cloudy coarse emulsion. From Figure (1) it is evident that the aqueous micellar solution containing no sugars (pure water micellar solution) was able to solubilize the largest load of IAA (110µl/10g micellar solution ~1.434 mmol/g SL). This value was declined to 80 µl, 70 µl and 60 µl (~1.044, 0.912 and 0.783 mmol/g SL) when the aqueous micellar solutions were composed of water/citric/sucrose, water/citric/fructose and water/citric/glucose, respectively. It is worth noting that the molecular solubility of IAA in pure water due to hydrogen bonding (i.e. without surfactant) is 1: 400 parts by weight at 25ºC, respectively [7]. This value is equivalent to 28.5µl IAA/10g pure water. Looking at the particle size of each of the four microemulsions which correspond to that amount of IAA in figure (1), one can see an increase in the micelle size relative to the zero load of IAA. This means that only fraction of this amount stayed in water while most of this value partitioned and solubilized into the micellar aggregates causing increase in the particle size. It may not be surprising to detect decline in the solubilized loads of IAA due to addition of sugars. These additives are known to change the micellization characteristics of the surfactant leading to decrease in the critical micelle concentration and increase in their aggregation number [8]. That affects ultimately on the number of micelles that are responsible for solubilization. In addition sugars are well known to have a salting out effect [9] that can decrease the amount of moleculare solubilization of IAA in sugar-containing microemulsions.

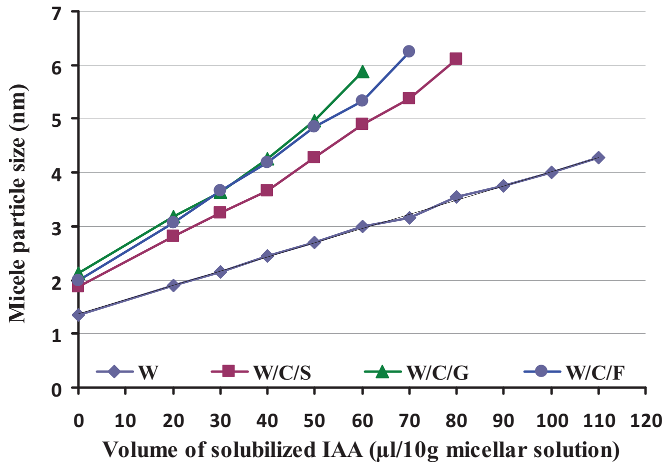

Fig. 1. Maximum solubilized loads of IAA incorporated in SL micellar solutions formulated with different aqueous solutions. (W) water; (C) citric acid; (S): sucrose; (G) glucose; (F) fructose

Based on the solubilization behavior shown in figure (1) it is evident that SL in pure water can produced more number of micelles compared with the numbers in the other micellar solutions containing sugars. What seem interesting is the deferential effect of the three sugars on solubilization of IAA and the particle size of microemulsion. From Figure (1) it is clear that the magnitude of solubilization of IAA in these sugars micellar solution were in the order: sucrose > fructose > glucose. A potential reason for that is based on the effect of the different sugars on water activity and the availability of "free water". Water activity describes the amount of water available for hydration or interaction with other solutes via hydrogen bonding. In case of pure water the water activity is maximized and equals 1.0. Thus there is large amount of non-interacted water "free water" available to interact with (i.e. hydrate) the hydrophilic head groups of the surfactant. That high degree of freedom in forming micelles can lead to increase in their number and consequently increase the maximum solubilized load of IAA. On the other hand when water interacts with sugars its water activity decreases depending on the sugar type [9] and how each sugar will interact via hydrogen bonding with water. That makes water become less available for other hydration interactions [10]. Thus the amount of "free water" in the sugar-containing systems decreases leading to smaller interaction with surfactant molecules and smaller number of micelles and hence smaller solubilization capacity. More over there could be some interactions that exist between the surfactant molecules and the different sugars in each system due to the presence of numerous hydroxyl groups in both of them that can form hydrogen bonds. That interaction will be on the expense of the interaction between surfactant with free water to form micelles. This can potentially leads to decrease the number of micelles available for solubilization.

In sugar-containing microemulsins, the aggregation number of micelles could increases due to jamming of surfactant molecules in order to fit into the small domain of available free water. That in turn can lead to increases the microemulsion particle size. The above mentioned justification could be confirmed from figure (2). The figure shows that the size distribution of microemulsion formulated with pure water is mono-modal up to 50µl of solubilized IAA. The particle size of that microemulsion was 2.70 nm and PDI was 0.18. On the other hand, microemulsions formulated with sugars showed bi-modal size distribution pattern at the same load of IAA (50µl). Their particle size increased to 4.28nm-4.97nm and the PDI also increase to ~ 0.3 and became less uniform. Previous investigations also confirmed that the aggregation number of surfactant micelle increased in the presence of *d*-glucose compared with pure water [11,12]. The increase in the micelle size results not only from the incorporation of the guest molecule but also because of the increasing number of surfactant molecules that are accommodated within a micelle [13].

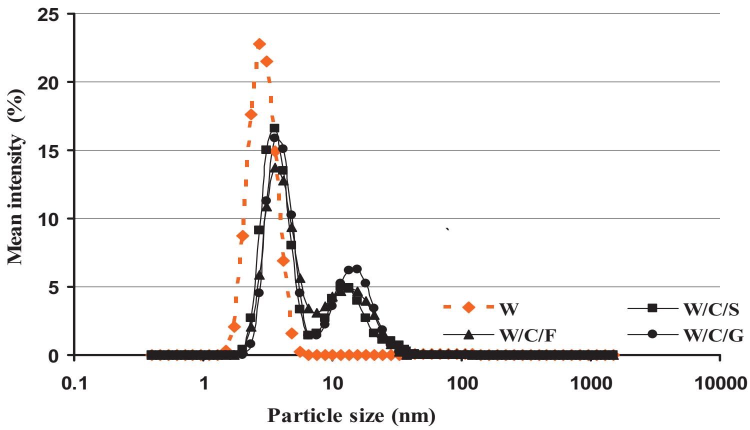

Fig. 2. Particle size distribution of microemulsions containing 50 µl IAA solubilized in 10g of 5.% SL micellar solutions formulated with different aqueous solutions. (W) water; (C) citric acid; (S): sucrose; (G) glucose; (F) fructose

Investigation of the dilutability of IAA microemulsions indicated that all the formulated systems can be diluted with the corresponding aqueous solution up to 10-folds without losing the nano-structure. The particle size of the microemulsions at the highest dilution level ranged between 9.5nm-16.0nm, PDI 0.12-0.18 depending on the type of sugar.

It was interesting to note that glucose and fructose solubilized slightly different amounts of IAA although both of them belong to the same category of mono-saccharides and have the same molecular formula ($C_6H_{12}C_6$). That can be rationalized on the basis of the small change in the stereo-chemical structure of their molecules. Glucose is an aldose sugar having aldehydic group on the terminal carbon atom while fructose is ketose that has ketonic group next to the terminal carbon atom (figure 3). That can change the micellization characteristics and the solubilization behavior of their micellar solutions towards IAA [14].

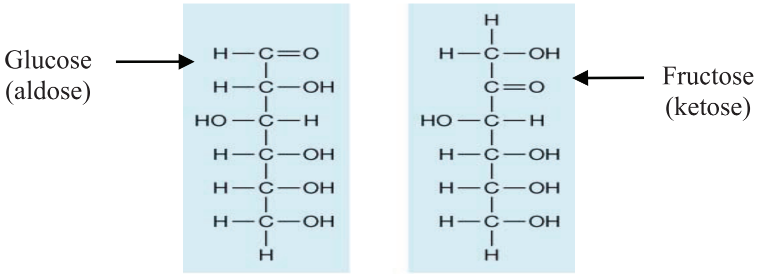

Fig. 3. Chemical structure of glucose and fructose

4. Conclusion

This paper is a trial to formulate water-based microemulsion system capable of delivering a model flavor ester like IAA without using organic solvents. The solubilized amounts of the ester depend mainly on the sugar composition of the aqueous micellar solution. The reader should bear in mind that IAA is just a single aroma impact ester which represents a "simple" banana flavor model. The real banana flavor formula is much more sophisticated than that and its solubilization characteristics will need further investigation. A complementary investigation to the present work is required to evaluate the effect of flavour solubilization in surfactant micelle on the sensory perception and aroma release from the final product.

Acknowledgements

The authors are indebted to the British Royal Society and Egyptian National Research Centre for providing financial support. Special thanks to Mitsubishi –Kagaku Foods Corporation, Japan for donating sucrose laurate sample.

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Presented at ICEF11 (May 22-26, 2011 – Athens, Greece) as paper FMS054.